Plant and Soil **205:** 51–56, 1998.

Effect of four acid soils on root growth of white clover seedlings using a soil-on-agar procedure

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Received 5 January 1998. Accepted in revised form 21 August 1998

Key words: acid-soil resistance, aluminum, soil solution, *Trifolium repens*

Abstract

White clover (*Trifolium repens* L.) is widely distributed in the Appalachian region, except on highly acid soils. We used a procedure where a thin layer of soil is placed on top of solidified water agar to characterize effects of acid soil on seedling root growth. Our objectives were to evaluate the soil-on-agar technique by using four soils (non-limed and limed) with diverse chemical characteristics and to relate root emergence to the chemical properties of the soils. We used three white clover cultivars, 'Grasslands Huia', 'Grasslands Tahora' and 'Sacramento'. Daily counts of root emergence from soil into agar were made for 12 d. Liming hastened white clover root emergence in three of the four soils. Days to 40% emergence were closely related ($P < 0.01$) to soil $pH_{(H_2O)}$ and to species of soil solution Al that are associated with Al toxicity in dicotyledonous plants. The r^2 values for the regression of days to 40% root emergence on $pH_{(H_2O)}$, Al^{3+}, $Al(OH)^{2+}$ and (Al^{3+} + $Al(OH)^{2+}$+ $Al(OH)_2^+$) were 0.95, 0.96, 0.94 and 0.96, respectively. Apparently, the primary factor responsible for delayed root emergence in the soil-on-agar procedure was Al toxicity. Because of the close relationship between root emergence and activity of toxic species of soil solution Al, we propose that the soil-on-agar technique should be useful for characterizing the response of many small-seeded species to Al.

Introduction

White clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) are important components of many Appalachian pastures but, they are rarely found on strongly acidic soils. When lime is applied to such soils these clover can become established and persist. However, because of rugged terrain and high costs, application of soil amendments are not always feasible. Thus, clover germplasm with increased acid-soil resistance has the potential to improve plant establishment and persistence on highly-acid soils.

Aluminum toxicity is a major factor limiting plant growth in acid soils. Response to toxic levels of Al is rapid (Kochian, 1995) and results in a dramatic reduction in root growth. Thus, short-term bioassays (Belesky et al., 1991; Karr et al., 1984; Ritchey et al., 1988) have been proposed to assess the toxicity of acid soils or evaluate Al resistance of plant germplasm. We have proposed a soil-on-agar procedure for assessing the acid-soil resistance of small-seeded legumes (Voigt et al., 1997). This procedure allows repeated (nondestructive) visual evaluation of root emergence from a soil layer into translucent water agar, over a period of several days. Different germplasm can be compared and/or individual seedlings can be easily identified and saved for further studies or development of new populations.

Our previous studies (Voigt et al., 1997) were conducted with one soil. The objectives of this study were to extend our technique to additional soils and to relate the results obtained to the chemical characteristics of those soils.

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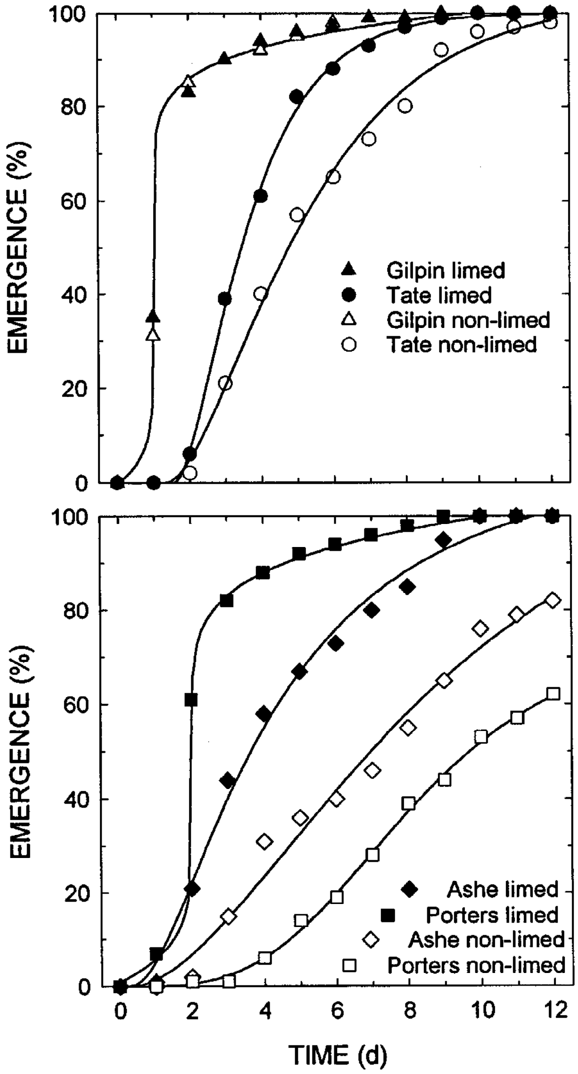

Figure 1. White clover root emergence from a layer of limed or non-limed Gilpin, Tate, Ashe or Porters soil, into agar over time. Curves fit are asymmetric transition functions, e.g., log normal cumulative or logistic dose. A single emergence curve was fit to the mean of the Gilpin limed and non-limed soils.

Materials and methods

Treatments and data collected

The evaluation technique has been described (Voigt et al., 1997). Briefly, a thin layer (about 8 mm) of moist soil is uniformly distributed on top of solidified water agar (5 g kg^{-1} agar in distilled water) in a rectangular, clear-plastic flask. Germinated seeds, selected for a uniform radical length of about 1 mm, are planted immediately below the soil surface. Flasks are then placed in a growth chamber for the duration of the experiment. Root emergence from the soil into the agar is observed visually at any desired time interval.

We used four Appalachian soils; Ashe (coarse-loamy, mixed, mesic Typic Dystrochrept), Gilpin (fine-loamy, mixed, mesic Typic Hapludult), Porters (coarse-loamy, mixed, mesic Umbric Dystrochrept) and Tate (fine-loamy, mixed, mesic Typic Hapludult); collected, prepared and analyzed for an unpublished study conducted in 1990. Lime ($Ca(OH)_2$) in the amounts needed for the unpublished experiment had been mixed with each soil (Table 1), based on the requirements of that study. Soils had been analyzed as described elsewhere (Baligar et al., 1991). Briefly, exchangeable Al was extracted by 1 *M* KCl while other bases were determined from extractions using 1 *M* NH_4OAc at pH 7. Determinations were by ICP emission spectroscopy. Soil solution was extracted by centrifugation (Reynolds, 1984). Formation constants for mononuclear Al species (Nordstrom and May, 1989) were used in a modified GEOCHEM computer program (Sposito and Mattigod, 1980) to evaluate activities of free ions in soil solution. The limed and non-limed soils varied widely in Al content and Al saturation (Table 1). All were relatively low in Mn content.

White clovers cultivars ‘Grasslands Huia’, ‘Grasslands Tahora’ and ‘Sacramento’ were used. Flasks, the experimental unit for the factorial experiment, contained 18 seedlings and were arranged in a randomized complete block design of six replications in a growth chamber (14 h light at 23 °C, 10 h dark at 17 °C). Light intensity in the chamber was 450μ mol m^{-2} s^{-1}, although light level perceived by the seedlings was slightly less because the flasks were covered with translucent plastic supported by a frame, to reduce moisture loss.

Root emergence, i.e., visible extension of the seedling’s root from the soil into the agar, was recorded daily for 12 d. The number of dead seedlings was counted at the end of the experiment.

Statistical analyses

Daily root emergence data were converted to a percentage of the number of germinated seed planted. Number of days to 40% emergence (E_{40}) was estimated for each flask using a fifth order polynomial regression of root emergence on days. For most flasks, this polynomial fit the data very closely within the 30–70% emergence range. Three of 180 flasks did

Table 1. Five Appalachian soils, amount of lime added to each, and their chemical properties[a]

Soil	Lime (g kg^{-1})	Al	Mn (cmol kg^{-1})	Ca	Mg	$pH_{(H_2O)}$	$Al_{(sat.)}$[b] (%)
Ashe	0	1.27	0.01	0.35	0.22	4.3	50
Ashe	0.77	0.09	0.01	3.45	0.21	4.6	2
Gilpin	0	1.13	0.26	2.02	0.91	5.1	23
Gilpin	0.31	0.29	0.22	3.28	0.90	5.5	6
Porters	0	3.74	0.02	0.07	0.06	4.1	84
Porters	1.54	0.26	0.01	6.33	0.06	5.2	4
Tate	0	2.97	0.07	0.45	0.20	4.4	74
Tate	1.22	1.00	0.09	0.91	0.21	4.8	39

[a] Soil chemical data courtesy of V C Baligar.
[b] Percent Al saturation = exch. Al/exch. (Al+Ca+Mg+K+Mn+H) × 100.

not reach 40% emergence during the study and values were assigned to those treatments by extrapolation from a graph of their emergence data.

Homogeneity of error among the ten soil–lime combinations was tested using Chi square. Transformations used to equalize error variance were arc sin (cumulative percent emergence) and log (E_{40}). Results are presented in the untransformed scale, but statistics are based on the transformed scale. For the overall analysis of variance (ANOVA) of cumulative root emergence, data for days 1 and 9 through 12 were deleted to minimize effects of non varying data, 0 or 100% emergence, on estimates of experimental error. Repeated measures analysis were used for all sources of variance that included day (SAS, 1988). The covariance structure of the repeated measures was of a non-spherical form that invalidated the usual ANOVA F tests for the day main effect and its interactions ($\chi^2_{df=20}$ = 663.93, $P < 0.0001$). Thus a multivariate analysis of variance (MANOVA) was used to analyze the day main effect and any interaction with day. Wilks' Λ statistics were used to determine significance of these effects.

Based on the initial ANOVA, soils were divided into non-responding and responding groups, i.e., soils where white clover root emergence did not or did respond to the amount of lime applied, respectively. For those two groups of soils, most emergence occurred from days 1 to 5 and 2 to 10, respectively. Data for the two groups of soils were analyzed separately using data from the above dates. This allowed inclusion of additional data while still minimizing non-varying (0 or 100%) data.

Soil chemical characteristics were related to cumulative root emergence (all 12 days) and E_{40}, using means calculated over replications and cultivars, by correlation and regression procedures.

Results

Soil had a striking effect on root emergence from the soil into the agar, i.e., root growth (Figure 1). Addition of lime altered this response for all soils except Gilpin. Root emergence could be divided into three stages. The initial stage was the delay in time before the first roots emerge, i.e., the time required for the roots that were growing the fastest to grow through the soil layer and into the agar. Time required for initial emergence varied from that of the limed or non-limed Gilpin soils, where 33% of the roots emerged on day one, to those of the other non-limed soils where less than 5% of the roots had emerged by day two. The second stage of emergence was when the majority of emergence occurred. The steeper the slope, during this relatively linear stage of emergence, the faster the rate of root emergence. Both Gilpin and the limed Porters soils were characterized by very rapid emergence. In contrast, the non-limed Ashe and Porters had the slowest rates of root emergence. The final stage was the slower emergence of many of the remaining roots and was characterized by a gradual plateauing as root emergence approached its maximum. Together, these stages gave a pattern of response that can be represented by the asymmetric, sigmoid type, curves used to characterize the data in Figure 1. The MANOVA of the cumulative root emergence data indicated that days and its interactions with soils, lime and the soils by lime interaction were all important ($P < 0.01$), con-

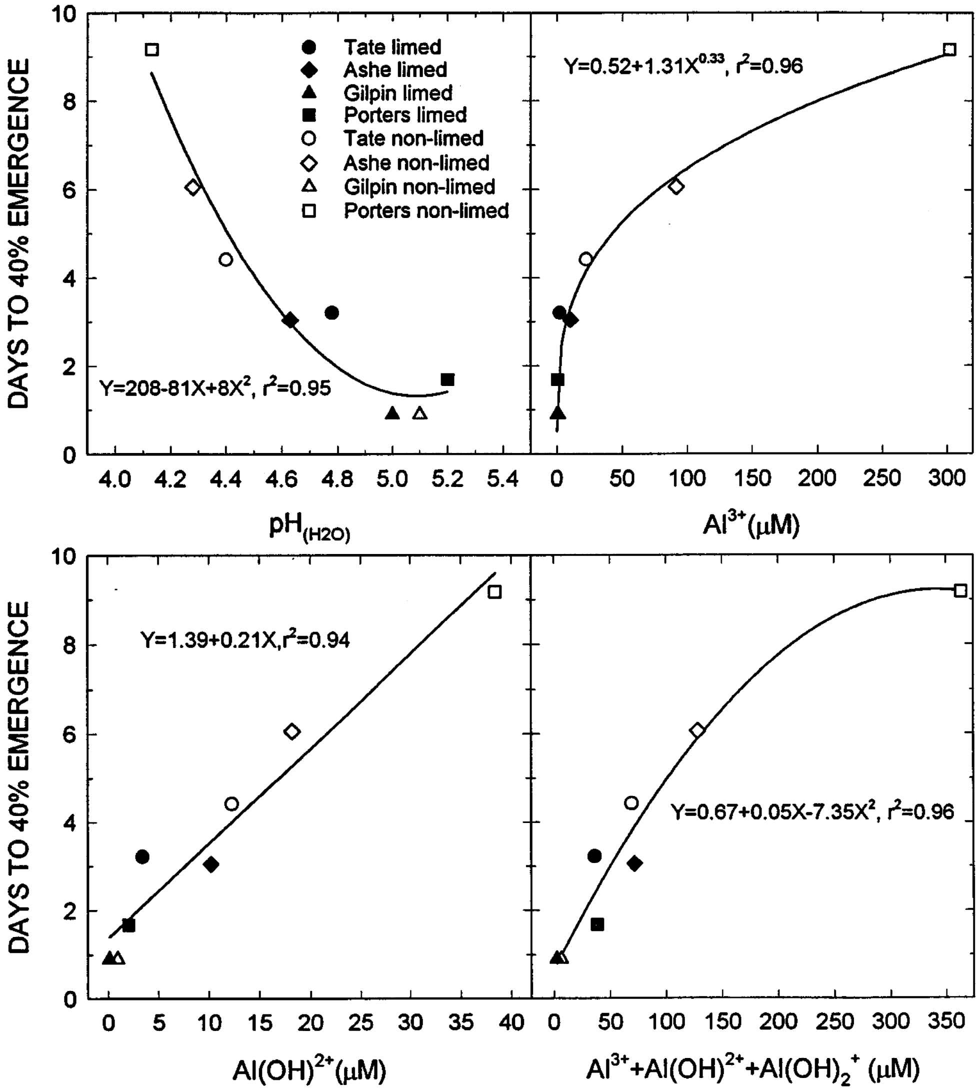

Figure 2. Effect of soil $pH_{(H_2O)}$ and species of soil solution (Al, Al^{3+}, $Al(OH)^{2+}$ and Al^{3+} + $Al(OH)^{2+}$ + $Al(OH)_2^+$) on time to 40% root emergence from limed and non-limed Ashe, Gilpin, Porters and Tate soil into agar.

firming the importance of the differences among these curves.

Averaged across days, differences among soils and between lime levels were important for both root emergence and E_{40} (Table 2). Interactions between soils and lime rates were important ($P < 0.01$). Here, also we were unable to detect a root emergence response to lime for the Gilpin soil. However, the non-limed Gilpin soil was higher in pH and Ca and lower in Al saturation than any other non-limed soil (Table 1). These values were such that a dramatic, short-term response from white clover might not be expected.

In contrast, white clover root emergence increased and E_{40} decreased when lime was applied to the Tate, Ashe and Porters soils (Table 2). When emergence data were reanalyzed, with soils grouped by the white clover response, an interaction was still detected between soil and lime rate for the responsive group ($P < 0.01$). Thus, the amount of white clover response to lime varied among these three soils.

Data on number of dead plants were not analyzed statistically because that data set contained mostly ze-

Table 2. Root emergence of three white clovers from four limed or non-limed soils

Lime	Non-responding soil[a]	Responding soils			
	Gilpin	Tate	Ashe	Porters	Mean
Cumulative root emergence (%)					
No	79 a	59 bA	41 bB	23 bC	41 b
Yes	79 a	74 aB	69 aC	90 aA	78 a
Mean	79	66 A	55 B	56 B	
E_{40} (d)					
No	0.9 aD	4.4 aC	6.1 aB	9.2 aA	6.5 a
Yes	0.9 aC	3.2 bA	3.0 bA	1.7 bB	2.7 b
Mean	0.9 B	3.8 A	4.6 A	5.4A	

[a] Root emergence and E_{40} did not or did show a response to the amount of lime applied. Means within columns followed by the same lower case letter and means within rows followed by the same upper case letter are not significantly different at the 0.05 probability level by Duncan's multiple range test. For cumulative root emergence only, responding and non-responding soils should not be compared because they were calculated over different time periods.

ros. Less than 1% of the seedlings, 23, died. However, almost 80% of the dead seedlings came from the Ashe soil and 78% of these were from the non-limed treatment. No other soil–lime treatment had more than two dead seedlings.

Linear correlation coefficient between cumulative emergence and E_{40} was r = –0.99 ($P < 0.01$). Because E_{40} is easier to visualize than cumulative emergence, results presented for intercharacter correlation coefficients and regressions will be based on E_{40}. Most correlation coefficients between E_{40} and soil characteristics were not significant ($P > 0.05$). The largest linear correlation coefficients were with soil solution Al, soil $pH_{(H_2O)}$, soil solution pH, base saturation and acidity, r = 0.93, –0.92, –0.88, –0.85 and 0.84 ($P < 0.01$), respectively. Additional correlation coefficients of moderate size were with, exchangeable H, exchangeable Al, exchangeable bases and Ca saturation, 0.80, 0.79, –0.78 and –0.72 ($P < 0.05$), respectively. Some curvilinear responses were detected. The quadratic response observed for $pH_{(H_2O)}$ was significant ($P < 0.05$) (Figure 2).

Because the activity of species of Al found in the soil solution are believed responsible for the toxic effect of Al on root growth (Kinraide, 1991; Kochian, 1995) we examined the relationship of Al species to root emergence in our experiment. Results from studies conducted in solution culture, e.g., Kinraide and Parker (1990) and Noble et al. (1988), and soil, Bruce et al. (1980) and Menzies et al. (1994) indicate that in dicotyledonous plants, Al^{3+} and $Al(OH)^{2+}$ may be associated with Al toxicity.

In our results, a linear relationship existed between Al^{3+} and E_{40}. Although the quadratic response was not significant ($P > 0.05$), a plot of the data suggested that the relationship between these two variables was curvilinear. A power equation fit the data points quite well (Figure 2). The $Al(OH)^{2+}$ species of Al had the closest linear relationship of any soil variable with E_{40} (Figure 2). A third Al species, $Al(OH)_2^+$, was not related to E_{40} (not shown). When these three species of Al were summed, the quadratic response of E_{40} was significant ($P < 0.05$) and resulted in a very close fit to the data points (Figure 2).

Discussion

Although the non-limed Porters soil data point appears to have a dramatic effect on some of the Al species E_{40} relationships (Figure 2), and it would be desirable to have data points between the non-limed Ashe soil and non-limed Porters data points, deletion of the non-limed Porters data point does not eliminate the relationship with E_{40}. The three relationships, between E_{40} and Al species, that were significant remain significant ($P < 0.01$) with r^2 of 0.68, 0.82 and 0.86 for Al^{3+}, $Al(OH)^{2+}$ and (Al^{3+} + $Al(OH)^{2+}$ + $Al(OH)_2^+$), respectively. All were linear. Thus, the relationship between E_{40} and Al toxicity remains strong even when the highly toxic Porters soil is not included.

We do not assume that the relationship observed here, between $Al(OH)^{2+}$ and E_{40} implies direct causation. The two forms of soil solution Al, Al^{3+} and $Al(OH)^{2+}$, are not independent, r = 0.95 ($P < 0.01$).

Thus, the relationship between $Al(OH)^{2+}$ and E_{40} is probably not direct or causative (Kinraide, 1991).

Our results indicate a very close relationship between root growth of white clover in the soil-on-agar system and the species of Al, found in the soil solution, that are associated with the toxicity of Al to other dicotyledonous plants, e.g., soybean, *Glycine max* (L.) Merr. (Bruce et al., 1988) and mungbean, *Vigna radiata* (L.) Wilczek (Menzies et al., 1994). Thus, our results support the hypothesis that the primary factor causing delayed root emergence in the soil-on-agar procedure is Al toxicity.

Of the four soils used in this study, the Porters soil appears to be the best soil for evaluation of white clover germplasm for Al resistance when using the soil-on-agar technique. The Gilpin soil was not toxic to white clover. High levels of stress were obtained with the Ashe soil, but it also accounted for most of the death loss among seedlings and there was a tendency for soil particles from the Ashe soil to slide a short distance down the inside of the flask, between the agar and the flask, thus making root observation more difficult. The Tate soil could be used for white clover, but might not be sufficiently toxic for maximum discrimination among the most Al resistant white clover seedlings.

The soil-on-agar procedure can detect differences in root growth among cultivars of white clover (Voigt et al., 1997). It appears to be a valid procedure for determining the seedling response of white clover germplasm to Al. The technique should be easy to extend to other small-seeded species.

Acknowledgement

We are indebted to Dr V C Baligar for providing the soils and the soil analysis data used in this study.

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Section editor: R J Haynes